GB938937

Publication Title:

Water-soluble vegetable oil sterol derivatives

Abstract:

Abstract of GB938937

The invention comprises polyethylene glycol esters of the formula <FORM:0938937/IV(a)/1> wherein S represents a phytosterol residue, R represents an alkylene radical having 2 to 6 carbon atoms and "PEG" represents a polyethylene glycol residue derived from a polyethylene glycol having an average molecular weight lying in the range of 400 to 6,000, and the preparation thereof by reacting a phytosterol with the anhydride of the required dicarboxylic acid and esterifying the resulting phytosterol acid ester with a suitable polyethylene glycol. Suitable dicarboxylic acids are succinic, glutaric, adipic, pimelic and suberic acids. The phytosterol residue may be derived from a vegetable oil such as soybean, corn, peanut or cotton seed oil-particularly sitosterol, stigmasterol and compesterol.ALSO:The invention comprises a polyethylene glycol ester of a phytosteryl acid ester of a dicarboxylic acid of the general formula <FORM:0938937/IV(a)/1> wherein S represents the phytosterol residue, R represents an alkylene radical having 2 to 6 carbon atoms and PEG represents a polyethylene glycol residue derived from a polyethylene glycol having an average molecular weight of 400 to 6000, and the preparation thereof by reacting the required dicarboxylic acid anhydride with a phytosterol to form a phytosteryl acid ester, and esterifying with a polyethylene glycol having an average molecular weight of 400 to 6000, e.g. in the range of 600 to 1500. The dicarboxylic residue may be derived from succinic, glutaric, adipic, pimelic or suberic acid. The phytosterol residue may be derived from vegetable oils such as soybean, corn, peanut or cottonseed oil, in particular sitosterol, stigmasterol and campesterol. Therapeutic compositions suitable for treating dermatomyositis and for lowering the cholesterol level in blood serum comprise aqueous solutions of the above polyethylene glycol esters. Data supplied from the esp@cenet database - Worldwide

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PATENT SPECIFICATION

NO DRAWINGS

938,937

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International Classification:—C07c (C08g).

COMPLETE SPECIFICATION

Water-Soluble Vegetable Oil Sterol Derivatives

We EASTMAN KODAK COMPANY, a Company organized under the Laws of the State of New Jersey, United States of America, of 343, State Street, Rochester 4, New York, United States of America, (Assignee of MAX HERMAN STERN), do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention concerns sterol derivatives, and more particularly, water-soluble vegetable oil sterol derivatives.

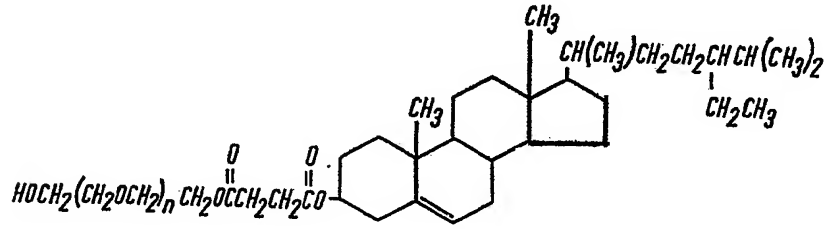
Vegetable oil sterols are commonly referred to as "phytosterols." Typical phytosterols include sitosterol, stigmasterol and campesterol, sitosterol being the phytosterol occurring in the largest proportions in vegetable oils. Such phytosterols as sitosterol have been employed in the diets of various animals including humans as an agent to lower the cholesterol level in the blood serum. Hypercholesterolemia can be substantially reduced with sterols and with sterol esters of short-chain fatty acids. However, sterol esters of long-chain fatty acids have little or no activity for reducing hypercholesterolemia.

The oral administration of sterols is complicated by their tendency to be unpalatable and nauseating. In addition, sterols are not water-soluble but rather oil-soluble compounds. It would be desirable to have a water-soluble phytosterol compound that could be administered orally in conjunction with various liquid foods such as fruit juices soups and beverages, as well as in conventional aqueous therapeutic preparations.

It is an object of the invention to provide new phytosterol derivatives.

The phytosterol derivatives of the invention can be represented by the following general formula

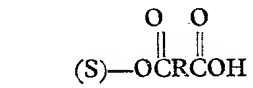
wherein (S) is phytosterol moiety for example derived from such vegetable oils as soybean oil, corn oil, peanut oil or cottonseed oil, wherein R is an alkylene radical having 2 to 6 carbon atoms; and wherein (PEG) is a polyethylene glycol moiety derived from a polyethylene glycol having a molecular weight in the range of 400 to 6000. A typical water-soluble sterol derivative of the invention is a polyethylene glycol ester of sitosteryl acid succinate having the following structure:



wherein n is an integer from 8 to 135.

[*Pr.*

The water-soluble phytosterol derivatives of the invention can be prepared by first preparing a phytosteryl acid ester of a dicarboxylic acid having the formula



wherein (S) and R are the phytosterol and alkylene substituents described above. The phytosterol acid ester of a dicarboxylic acid can be prepared by any of the well-known procedures for making acid esters of hydroxylic compounds, a preferred method being to react the phytosterol with a suitable dicarboxylic acid anhydride under the usual esterification conditions as illustrated in the specific examples given below. The dicarboxylic acid moiety of the compounds of the invention can be derived from such acids as succinic acid, glutaric acid, adipic acid, pimelic acid and suberic acid.

The resulting phytosterol acid esters are then esterified with polyethylene glycol in accordance with well-known esterification techniques. The esterification may be effected in an inert organic solvent medium such as toluene, xylene, petroleum ethers, benzene naphtha or other solvents which do not react or esterify with the reactants. The esterification can be promoted with catalytic amounts of such esterification catalysts as *p*-toluene sulphonic acid, sulphuric acid, trichloro-acetic acid, oxalic acid or hydrochloric acid. The reaction is desirably effected at an elevated temperature such as 60°—150°C. or at reflux temperatures of the solvent, with the water formed during the reaction being removed azeotropically during the course of the esterification. The polyethylene glycol employed desirably has an average molecular weight of at least 600 to give products having good water solubility, although polyethylene glycols having molecular average weights as low as 400 can be employed where lower water solubilities are suitable. Highly water-soluble products are obtained with polyethylene glycols having average molecular weights up to 6000. In order to obtain highly potent phytosterol preparations without too high a percentage of polyethylene glycol moiety, however, the polyethylene glycol desirably has an average molecular weight not substantially higher than about 2000, with polyethylene glycols having average molecular weights in the range of 600 to 1500 being preferred.

Most commercially available polyethylene glycol compositions are mixtures of materials of varying molecular weights and are sold on a basis of the average molecular weight of the composition. Best results in preparing the phytosterol derivatives of the invention are obtained by distilling, solvent extracting or otherwise separating the low molecular weight components of such mixtures from the bulk of the mixture since components below a molecular weight of about 400 lessen the water solubility of the final ester. Thus, for example, it is desirable to distil a polyethylene glycol composition having an average molecular weight of about 1000 to remove as much as 15% by weight of the low boiling components, such distillations being readily effected in high vacuum, short path, centrifugal stills. Alternatively, the commercial polyethylene glycol compositions can be employed directly to esterify phytosteryl acid esters of dicarboxylic acids and the resulting product solvent extracted with petroleum ether or a mixture of solvents such as benzene and petroleum ether, for example, to extract out the less water-soluble components, or the desired product can be crystallized out of a suitable solvent such as ethanol or isopropanol.

The phytosterol derivatives of the invention can be employed to form clear aqueous solutions containing as much as 25% or more by weight of the phytosterol derivative. Such aqueous solutions can be utilized in various therapeutic preparations. For example, the stigmasterol derivatives of the invention are deemed good water-soluble sources of stigmasterol, which sterol is used in the treatment of dermatomyositis as described in Arch. Biochem. *1951,* 326. In addition, the phytosterol derivatives of the invention are deemed good water-soluble sources of phytosterols such as sitosterol which is used to reduce hypercholesterolemia or cholesterol in mammal blood serum. This latter property of the phytosterol derivatives of the invention was quite unexpected as several workers in this field have shown that, while phytosterol and phytosterol esters of short-chain fatty acid esters such as the formate ester and the acetate ester were effective in reducing hypercholesterolemia, phytosterol esters with long-chain fatty acids such as the palmitate ester and the oleate ester had very little or substantially none of such activity. However, the water solubilizing "tail-piece" on the active phytosterol derivatives of the invention is not a short-chain moiety, but is rather a long-chain, high molecular weight moiety.

The preparation of phytosterol derivatives embodying the invention is illustrated by the following examples of preferred embodiments, it being understood that similar results are obtained by the esterification of any of the phytosterol acid esters of dicarboxylic acids with other polyethylene glycols as described above.

EXAMPLE 1

A solution of 149 g. of mixed soybean sterols (97% sterols by the Liebermann-Burchard reaction consisting essentially of about 70% sitosterol, about 25% stigmasterol and about 5% other phytosterol material including campesterol) in 500 cc. of benzene was dried azeotropically by distilling off 400 cc. of benzene. Then 270 cc. of potassium hydroxide-dried pyridine and 47.5 g. of succinic anhydride were added, a condenser (capped with a calcium chloride drying tube) attached in a reflux position, and the reactants heated on a steam bath for 3 hours. After cooling to 25—30°C., 500 cc. of diethyl ether was added and the reaction mixture stored at 5°C. for 2 hours to precipitate unreacted succinic anhydride. The precipitated succinic anhydride was removed by filtration and the resulting filtrate washed with 5% hydrochloric acid and water to neutrality. After drying over anhydrous sodium sulphate, the solvent was removed by distillation to give 173.6 g. of soybean steryl acid succinate as a light tan solid having a neutralization equivalent of 537 and an infrared spectrum indicating the absence of free hydroxyl groups.

A 152 g. portion of the soybean steryl acid succinate prepared as described above 304 g. of polyethylene glycol having an average molecular weight of about 1000 ("Carbowax 1000" — Registered Trade Mark), and 3 litres of toluene were heated to 50°C. in a 5 litre round bottom flask equipped with a stirrer, thermometer, azeotrope tube and calcium chloride drying tubes. Then 1.88 cc. of concentrated sulphuric acid was added to the flask and the contents refluxed for 2 hours with stirring, 7.0 cc. of water being collected in the azeotrope tube. The resulting reaction mixture was cooled to 25—30°C., stirred vigorously for 30 minutes with 30 g. of potassium carbonate, and then 2 more hours with 150 g. of magnesium oxide ("Seasorb 43"). The resulting solution was filtered through a layer of diatomaceous earth ("Celite" — Registered Trade Mark) and the solvent removed by vacuum distillation. The resulting filtrate was purified by successive extractions with 4 litre portions of petroleum ether (boiling range 40—60°C), and with 4 litres of a 90/10 petroleum ether/benzene mixture. Each extraction was carried out with vigorous stirring for 25 minute periods. Following each extraction the supernatant phase was decanted and the residual suspension centrifuged to remove excess solvent. The extracted product (324 g.) was dissolved in 2.4 litres of ethanol, crystallized at 5°C., filtered at 5°C. and freed of solvent by evacuation for 16 hours at 5°C. in a desiccator containing calcium chloride. The resulting composition of polyethylene glycol ester of an acid succinate of soybean sterols was found to have the following properties:

a) Liebermann-Burchard reaction corresponding to 22% soybean sterols,
b) percent unsaponifiable fraction = 20.1,
c) Liebermann-Burchard reaction on unsaponifiables = 97.2%,
d) infrared spectrum showed substantially 100% ester, and
e) one part by weight soluble in three to four parts by weight of water at 25°C.

The water-soluble polyethylene glycol ester of an acid succinate of soybean sterols prepared as described above was tested for its hypocholesterolemic effect in rats. The rats were fed *ad libitum* for 4 weeks on a basal diet with various additives as summarized in the table below. The cholesterol in the blood serum after 4 weeks is also set out in the table below for the rats fed on the various diets.

TABLE

Group	Diet	Blood Serum, Total Cholesterol (mg.%)
13	Basal alone	220.6
11	Basal with 1% cholesterol	705.2
22	Basal with 1% cholesterol and 1% soybean sterols	388.5
28	Basal with 1% cholesterol and 1%* of the prepared soybean sterol derivative	377.1

*(based on the weight of the sterol moiety of the derivative). It is particularly noteworthy that the present water-soluble soybean sterol derivative, even though it contains a long-chain moiety, is very effective in reducing the cholesterol content in blood serum. The basal diet utilized to feed the rats was composed of approximately 44.7% sucrose, 25% lard, 20% "Vitamin Test" casein, 5% U.S.P. Salt XIV (page 789, 14th Ed. U.S.P.), 4% purified alpha cellulose, 1% cholic acid, 13% choline hydrochloride and small amounts of the fat-soluble and water-soluble vitamins. The vitamin content per 1 kilogram of diet comprised 20 mg. vitamin B_1 hydrochloride, 20 mg. vitamin B_2, 20 mg. vitamin B_6, 60 mg. calcium pantothenate, 100 mg. niacin, 2 g. choline, 1 g. inositol, 10 mg. 2-methyl-1,4-naphthoquinone, 200 mg. *p*-aminobenzoic acid, .4 mg. biotin, 4 mg. folic acid, .04 mg. vitamin B_{12}, 40,000 units vitamin A, 4000 units vitamin D and 335 units vitamin E.

EXAMPLE 2

A 2.0 g. portion of soybean steryl acid succinate prepared by the method described in Example 1 and 24 g. of polyethylene glycol having an average molecular weight of 6000 ("Carbowax 6000") in one litre of toluene in the presence of 0.3 g. of *p*-toluene sulphonic acid were reacted by the method described in Example 1. The resulting composition of polyethylene glycol ester of an acid succinate of soybean sterols was soluble in water. A clear aqueous solution containing 20% of the resulting soybean sterol derivative can be prepared.

All proportions of components referred to herein are on a weight basis.

The invention as illustrated by the Examples thus provides novel water-soluble phytosterol derivatives that are useful in therapeutic preparations.

WHAT WE CLAIM IS:—

1. A water-soluble polyethylene glycol ester of a phytosterol acid ester of a dicarboxylic acid having the formula

$$(S)\text{—}O\overset{O}{\overset{\|}{C}}R\overset{O}{\overset{\|}{C}}O\text{—}(PEG)$$

wherein (S) is a phytosterol moiety, R is an alkylene radical having 2 to 6 carbon atoms and (PEG) is a polyethylene glycol moiety derived from a polyethylene glycol having an average molecular weight lying in the range of 400 to 6000.

2. A water-soluble ester as claimed in claim 1 wherein the phytosterol moiety is a mixture of soybean sterols.

3. A water-soluble ester as claimed in claim 1 wherein the phytosterol moiety is sitosterol.

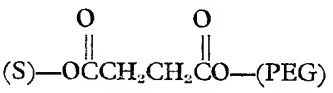
4. A water-soluble ester as claimed in claim 1 wherein the phytosterol moiety is stigmasterol.

5. A water-soluble polyethylene glycol ester of a phytosteryl acid ester of succinic acid having the formula:

$$(S)\text{—}O\overset{O}{\overset{\|}{C}}CH_2CH_2\overset{O}{\overset{\|}{C}}O\text{—}(PEG)$$

wherein (S) is a phytosterol moiety and (PEG) is a polyethylene glycol moiety derived from a polyethylene glycol having an average molecular weight lying in the range of 400 to 6000.

6. A water-soluble polyethylene glycol ester of a phytosterol acid ester of succinic acid having the formula



wherein (S) is a phytosterol moiety and (PEG) is a polyethylene glycol moiety derived from a polyethylene glycol having an average molecular weight lying in the range of 600 to 1500.

7. A water-soluble polyethylene glycol ester as claimed in claim 1 and as hereinbefore described with reference to either of Examples 1 and 2.

8. A method of making a polyethylene glycol ester as claimed in claim 1, which comprises reacting an anhydride of a dicarboxylic acid of the formula

wherein R has the meaning given in claim 1, with a phytosterol to obtain a phytosteryl acid ester, and esterifying the phytosteryl acid ester with a polyethylene glycol having an average molecular weight lying in the range of 400 to 6000.

9. A method of making a polyethylene glycol ester as claimed in claim 1 and substantially as hereinbefore described with reference to Example 1.

10. A method of making a polyethylene glycol ester, as claimed in claim 1 and substantially as hereinbefore described with reference to Example 2.

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